

Supporting Information:

Quantitative Profiling Method for Oxylipin Metabolome by Liquid
Chromatography Electrospray Ionization Tandem Mass
Spectrometry

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Table S-1. The Calibration Standards Concentrations for the 49 Oxylipins

analytes	1/30S1	1/10S1	1/3S1	S1	S2	S3	S4	S5	S6
6k PGF1a d4	0.02	0.06	0.2	0.60	2	10	20	100	1000
6k PGF1a	0.02	0.06	0.2	0.60	2	10	20	100	1000
TXB2	0.02	0.06	0.2	0.60	2	10	20	100	1000
9-12-13 TriHOME	0.02	0.06	0.2	0.60	2	10	20	100	1000
9-10-13 TriHOME	0.02	0.06	0.2	0.60	2	10	20	100	1000
PGF2a	0.1	0.3	1	3.00	10	50	100	500	5000
PGE2-d4	0.02	0.06	0.2	0.60	2	10	20	100	1000
PGE2	0.02	0.06	0.2	0.60	2	10	20	100	1000
PGD2	0.02	0.06	0.2	0.60	2	10	20	100	1000
11 12 15 THET	0.01	0.03	0.1	0.30	1	5	10	50	500
Lipoxin A4	0.02	0.06	0.2	0.60	2	10	20	100	1000
PGB2/PGJ2	0.07	0.21	0.7	4.2	7	14	70	140	1400
THF Diols	0.01	0.03	0.1	0.30	1	5	10	50	500
LTB4	0.02	0.06	0.2	0.60	2	10	20	100	1000
12 13 DHOME	0.2	0.6	2	6.00	20	100	200	1000	10000
10 11 DHHep	0.02	0.06	0.2	0.60	2	10	20	100	1000
9 10 DHOME	0.2	0.6	2	6	20	100	200	1000	10000
14 15 DHET	0.02	0.06	0.2	0.60	2	10	20	100	1000
11 12 DHET	0.02	0.06	0.2	0.60	2	10	20	100	1000
8 9 DHET	0.02	0.06	0.2	0.60	2	10	20	100	1000
15 deoxy PGJ2	0.04	0.12	0.4	1.2	4	20	40	200	2000
19 HETE	0.01	0.03	0.1	0.30	1	5	10	50	500
20 HETE d6	0.02	0.06	0.2	0.60	2	10	20	100	1000

20 HETE	0.01	0.03	0.1	0.30	1	5	10	50	500
5 6 DHET	0.02	0.06	0.2	0.60	2	10	20	100	1000
13 HODE	0.1	0.3	1	3.0	10	50	100	500	5000
9 HODE d4	0.02	0.06	0.2	0.60	2	10	20	100	1000
9 HODE	0.1	0.3	1	3.0	10	50	100	500	5000
10 11 DHN	0.02	0.06	0.2	0.60	2	10	20	100	1000
15 HETE	0.1	0.3	1	3.0	10	50	100	500	5000
13 oxo ODE	0.01	0.03	0.1	0.30	1	5	10	50	500
11 HETE	0.1	0.3	1	3.0	10	50	100	500	5000
9 oxo ODE	0.01	0.03	0.1	0.30	1	5	10	50	500
12 HETE	0.1	0.3	1	3.0	10	50	100	500	5000
8 HETE	0.1	0.3	1	3.0	10	50	100	500	5000
9 HETE	0.1	0.3	1	3.0	10	50	100	500	5000
5 HETE d8	0.02	0.06	0.2	0.60	2	10	20	100	1000
5 HETE	0.1	0.3	1	3.0	10	50	100	500	5000
12 13 EpOME	0.02	0.06	0.2	0.60	2	10	20	100	1000
14 15 EET	0.01	0.03	0.1	0.30	1	5	10	50	500
9 10 EpOME	0.02	0.06	0.2	0.60	2	10	20	100	1000
11 12 EET d8	0.02	0.06	0.2	0.60	2	10	20	100	1000
11 12 EET	0.01	0.03	0.1	0.30	1	5	10	50	500
8 9 EET	0.01	0.03	0.1	0.30	1	5	10	50	500
5 6 EET	0.01	0.03	0.1	0.30	1	5	10	50	500
15 oxo ETE	0.01	0.03	0.1	0.30	1	5	10	50	500
5 oxo ETE	0.01	0.03	0.1	0.30	1	5	10	50	500

Table S-2. LC Gradient Employed in the Current Study

total time(min)	flow rate(μ l/min)	B (%)
0.00	400	15
0.75	400	15
1.50	400	30
3.50	400	47
5.00	400	54
6.00	400	55
10.50	400	60
15.00	400	70
16.00	400	80
17.00	400	100
19.00	400	100
19.30	400	15
21.00	400	15

Table S-3. Optimized Mass Spectrometric Parameters

parameters	value
CUR:	20 psi
TEM:	550 °C
GS1:	50 psi
GS2:	30 psi
ihe:	ON
CAD:	High
IS:	-4500
DP	-60
EP	-10

Table S-4. Optimized Mass Transitions and Collision Energy for All the 49 Oxylipins

compound	period#	parent ion	product ion	CE(eV)
6-keto-d4 PGF1 α	1	373.3	167.1	38
6-keto-PGF1 α	1	369.3	163.2	36
TXB2	1	369.2	169.1	25
9,12-13-TriHOME	1	329.2	211.1	28
9,10-13-TriHOME	1	329.2	171.1	32
PGF2 α	1	353.2	309.3	28
PGE2-d4	1	355.3	275.3	27
PGE2	1	351.2	271.3	28
PGD2	1	351.2	271.3	26
11,12,15 THET	1	353.2	167.1	32
Lipoxin A4	1	351.2	115.2	20
PGB2/PGJ2	1	333.2	235.3	28
THF Diols	1	353.2	167.1	32
CUDA	2	339.2	214.3	33
LTB4	2	335.2	195.1	23
12,13-DHOME	2	313.2	183.2	32
10,11-DHHeP	2	301.2	283.3	32
9,10-DHOME	2	313.2	201.2	30
14,15-DHET	2	337.2	207.1	24
11,12-DHET	2	337.2	167.1	28
8,9-DHET	2	337.2	127.1	30
15-deoxy PGJ2	2	315.2	271.3	20
19-HETE	2	319.2	275.1	24
20-HETE-d6	2	325.2	281.2	51
20-HETE	2	319.2	275.2	23
5,6-DHET	2	337.2	145.1	26
13-HODE	2	295.2	195.0	25
9(S)-HODE-d4	2	299.2	172.0	28
9-HODE	2	295.2	171.0	25
10,11-DHN	2	329.2	311.4	33
15-HETE	2	319.2	301.4	18
13oxo ODE	2	293.2	113.0	29
11-HETE	3	319.2	167.2	23
15-oxo-EET	3	317.2	113.1	24
9-oxo ODE	3	293.2	185.1	28
12HETE	3	319.2	179.2	20
8-HETE	3	319.2	301.2	17
9-HETE	3	319.2	123.1	20

5-HETE-d8	3	327.2	116.1	24
5-HETE	3	319.2	115.1	21
12(13)-EpOME	3	295.2	195.2	20
14(15)-EET	3	319.2	219.3	18
9(10)-EpOME	3	295.2	171.1	22
11(12)-EET-d8	3	327.2	171.2	20
11(12)-EET	3	319.2	167.0	20
5-oxo-EET	3	317.2	273.2	20
8(9)-EET	3	319.2	123.0	20
<u>5(6)-EET</u>	<u>3</u>	<u>319.2</u>	<u>191.0</u>	<u>20</u>

Table S-5. Recovery in Spiked Quality Control Samples

analyte	conc. nM	QC1 mean recovery	recovery SD	QC2 mean recovery	recovery SD	QC3 mean recovery	recovery SD	QC4 mean recovery	recovery SD
6k PGF1a d4	0.40	N.D. ^a	N.D.	2	119%	11%	20	100%	1%
6k PGF1a	0.40	N.D.	N.D.	2	100%	35%	20	104%	1%
TXB2	0.40	108%	7%	2	102%	6%	20	108%	3%
9-12-13 TriHOME	0.40	o.r. ^b	o.r.	2	116%	7%	20	109%	12%
9-10-13 TriHOME	0.40	o.r.	o.r.	2	91%	5%	20	102%	17%
PGF2a	2.00	108%	20%	10	116%	12%	100	112%	9%
PGE2-d4	0.40	116%	25%	2	105%	10%	20	94%	2%
PGE2	0.40	113%	23%	2	115%	15%	20	101%	8%
PGD2	0.40	108%	24%	2	111%	18%	20	101%	4%
11 12 15 THET	0.20	N.D.	N.D.	1	102%	6%	10	98%	12%
Lipoxin A4	0.40	127%	22%	2	109%	22%	20	95%	8%
PGB2/PGJ2	1.40	o.r.	o.r.	7	91%	9%	70	108%	1%
THF Diols	0.20	N.D.	N.D.	1	118%	11%	10	102%	6%
LTB4	0.40	N.D.	N.D.	2	80%	13%	20	101%	3%
12 13 DHOME	4.00	118%	15%	20	109%	1%	200	104%	1%
10 11 DHHep	0.40	108%	8%	2	104%	5%	20	101%	2%
9 10 DHOME	4.00	119%	5%	20	112%	5%	200	105%	2%
14 15 DHET	0.40	108%	81%	2	84%	4%	20	96%	6%
11 12 DHET	0.40	105%	45%	2	94%	8%	20	96%	6%
8 9 DHET	0.40	111%	11%	2	98%	7%	20	92%	7%
15 deoxy PGJ2	0.80	115%	15%	4	114%	5%	40	125%	4%
19 HETE	0.20	N.D.	N.D.	1	N.D.	N.D.	10	86%	14%
20 HETE d6	0.40	N.D.	N.D.	2	N.D.	N.D.	20	102%	16%
20 HETE	0.20	N.D.	N.D.	1	N.D.	N.D.	10	94%	4%

5 6 DHET	0.40	96%	12%	2	87%	11%	20	96%	8%	200	102%	5%
13 HODE	2.00	116%	17%	10	107%	8%	100	101%	1%	1000	93%	4%
9 HODE d4	0.40	N.D.	N.D.	2	117%	9%	20	98%	3%	200	97%	4%
9 HODE	2.00	118%	5%	10	118%	4%	100	106%	4%	1000	96%	3%
10 11 DHN	0.40	106%	9%	2	93%	13%	20	101%	1%	200	99%	2%
15 HETE	2.00	117%	16%	10	108%	15%	100	102%	7%	1000	99%	7%
13 oxo ODE	0.20	N.D.	N.D.	1	N.D.	N.D.	10	120%	5%	100	98%	6%
11 HETE	2.00	124%	10%	10	116%	8%	100	110%	9%	1000	98%	4%
9 oxo ODE	0.20	N.D.	N.D.	1	N.D.	N.D.	10	102%	3%	100	92%	3%
12 HETE	2.00	116%	10%	10	114%	9%	100	107%	8%	1000	104%	6%
8 HETE	2.00	122%	6%	10	112%	5%	100	104%	9%	1000	101%	4%
9 HETE	2.00	113%	9%	10	106%	9%	100	98%	8%	1000	96%	4%
5 HETE d8	0.40	117%	8%	2	107%	14%	20	89%	7%	200	87%	3%
5 HETE	2.00	118%	12%	10	112%	11%	100	97%	8%	1000	92%	3%
12 13 EpOME	0.40	122%	13%	2	113%	18%	20	120%	3%	200	117%	10%
14 15 EET	0.20	N.D.	N.D.	1	116%	16%	10	87%	5%	100	88%	11%
9 10 EpOME	0.40	125%	17%	2	106%	15%	20	109%	3%	200	105%	7%
11 12 EET d8	0.40	113%	15%	2	98%	14%	20	94%	6%	200	98%	9%
11 12 EET	0.20	110%	16%	1	106%	9%	10	103%	6%	100	99%	8%
8 9 EET	0.20	114%	6%	1	117%	10%	10	97%	7%	100	96%	7%
5 6 EET	0.20	N.D.	N.D.	1	o.r.	o.r.	10	110%	24%	100	95%	5%
15 oxo ETE	0.20	118%	12%	1	107%	14%	10	96%	1%	100	93%	7%
5 oxo ETE	0.20	N.D.	N.D.	1	N.D.	N.D.	10	110%	10%	100	107%	12%

^a N.D. the concentration is below of LOQ. ^b o.r. the concentration is out of the linear range.

Table S-6. Internal Standards Used in the Current Method

	deuterated internal standards	synthetical compounds	time to add	aim
Type I Internal Standards	6k PGF1a d4 PGE2-d4 20 HETE d6 9 HODE d4 5 HETE d8 11,12 EET d8	10,11 DHHep 10,11 DHN	before extraction	to mimic the extraction of prostaglandins, diols, epoxides and other oxylipins.
Type II Internal Standards		CUDA	at the last step before analysis when reconstitute sample solutions	to account for changes in volume and instrument variability